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Exclusion of Linkage Between Schizophrenia and the Gene Encoding a Neutral Amino Acid Glutamate/Aspartate Transporter, SLC1A5

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An abnormality in glutamatergic function has been hypothesized as being of etiological importance in schizophrenia. Twenty-three multiplex English and Icelandic schizophrenia families were genotyped with a polymorphic dinucleotide repeat sequence in the 3′-untranslated region of the glutamate/aspartate transporter gene called SLC1A5. Using the lod and a model-free method of linkage analysis (MFLINK), no evidence of linkage between SLC1A5 and schizophrenia was found. Our results do not support the hypothesis that SLC1A5 gene mutations or allelic variants provide a major gene contribution to the etiology of schizophrenia. However, because of the likelihood of heterogeneity of linkage in schizophrenia, there is a case for testing other pedigrees for linkage to the SLC1A5 locus. The SLC1A5 locus is one of a complex family of genes encoding neutral amino acid transporter proteins and the genetic relation between these other loci and schizophrenia has not yet been established. *Am. J. Med. Genet. 74: 50–52, 1997.*

KEY WORDS: schizophrenia; glutamate hypothesis; human neutral amino acid transporter SLC1A5; lod linkage analysis; model-free linkage analysis

INTRODUCTION

Family, twin, and adoption studies in several populations have consistently implicated genetic susceptibility as a major etiological factor in schizophrenia [Tsuang et al., 1993]. There is increased awareness that neurotransmitters other than dopamine may be involved in the etiology of schizophrenia. The glutamate hypothesis of schizophrenia is partly based upon the psychotomimetic effects of phencyclidine (PCP), sometimes known as "angel dust." Phencyclidine, a noncompetitive antagonist of the NMDA subtype of glutamate receptors, produces a schizophreniform psychosis which may be characterized by both negative and positive symptoms of schizophrenia, including catatonia, hebephrenia, and schizophrenic thought disorder [Javitt and Zukin, 1991]. The glutamate hypothesis proposes that endogenous dysfunction of glutamatergic neurotransmission and consequently a disturbed balance between glutamatergic and other neurotransmitter systems such as dopamine and GABA, etc. in the brain might contribute to the pathogenesis of schizophrenia [Javitt and Zukin, 1991; Olney and Farber, 1995]. Preliminary findings of a regionally specific lowering of the expression of both NMDA and non-NMDA glutamate receptor genes have been reported in the postmortem brain tissue of schizophrenics [Eastwood et al., 1995; Breese et al., 1995; Deakin et al., 1989]. In addition, the potential excitotoxic effect of glutamate during brain development could provide a possible explanation for the anatomical brain abnormalities in schizophrenics such as the increase in volume of cerebral sulci and ventricles as well as a reduction in medial temporal lobe structures [Brown et al., 1986].

The excitatory signal transmitted by glutamate or other excitatory amino acids is generally removed by reuptake into the presynaptic terminals and surrounding glia cells by high-affinity glutamate transporter systems. A deficiency in uptake has been implicated in the pathogenesis of ischaemic brain damage

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[Kuwahara et al., 1992] and may also be involved in certain neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), in which Rothstein et al. [1995] found a selective reduction in the density of high-affinity glutamate transporter in autopsy tissue from brains in patients with ALS. Several related sodium-coupled glutamate/aspartate transporters, EAAC1, GLAST, GLT-1 have been discovered recently [Kanai et al., 1994]. The precise function of the high-affinity glutamate transporters has yet to be characterized, but their genes might all be candidates for increasing genetic liability to schizophrenia.

In the present study, we report our assessment of linkage between schizophrenia and a dinucleotide repeat polymorphism of the gene encoding a neutral amino acid transporter called SLC1A5, which is a member of this high affinity glutamate transporter family and has been localized to human chromosome band 19q13.3 [Jones et al., 1994].

MATERIALS AND METHODS

Family Sampling and Diagnostic Procedures

Twenty-three pedigrees (11 Icelandic and 12 English) containing cases of schizophrenia were studied. Subjects were interviewed using the Lifetime Version of the Schizophrenia and Affective Disorders Schedule SADS-L [Spitzer and Endicott, 1978]. This information was supplemented by material from case notes. Extensive tracing of pedigrees was carried out and attempts were made to characterize as far as possible the diagnoses of other members of the kindreds. Two psychiatrists who were blind to genotyping assigned consensus diagnoses according to Research Diagnostic Criteria, RDC [Spitzer et al., 1978]. Subjects were also rated for schizoid personality and schizotypal disorder using DSM-IIIR criteria. Pedigrees were included on the basis of containing multiple cases of schizophrenia, but no cases of bipolar affective illness and of appearing to demonstrate unilineal inheritance.

Two affection classes were used for the linkage analysis: "Core schizophrenia," denoted DOMS, consists of all psychotic categories including schizophrenia and unspecified functional psychosis; "schizophrenia spectrum," denoted DOMSS, consists additionally of schizoid and schizotypal personality disorder according to DSM-IIIR criteria and schizotypal features according to the RDC. Of the 375 individuals in these 23 pedigrees, 95 fell into the DOMS category and an additional 18 fell into the DOMSS category.

Genotyping

Genomic DNA was extracted from blood samples obtained from interviewed members. The polymerase chain reaction (PCR) was carried out as previously described [Kalsi et al., 1995] with oligonucleotide primers which detect a dinucleotide repeat polymorphism in the 3′-untranslated region of the SLC1A5 gene [Jones et al., 1994]. PCR conditions were denaturation at 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. Amplified PCR products were separated by size on a 6% polyacrylamide gel and visualized by autoradiography. The genotypes were read by two independent assessors blind to diagnostic data. Discrepant genotypes were discussed and a consensus agreed for linkage analysis. Seven alleles were noted in these 23 families with a heterozygosity of 0.814 and PIC (polymorphic information content) value of 0.750.

Linkage Analysis

Linkage analyses were carried out using FASTLINK [Lathrop et al., 1985; Cottingham et al., 1993; Schaffer et al., 1994] and MFLINK [Curtis and Sham, 1995]. For the DOMS model the penetrance for those carrying for the disease allele was set to 0.73, while sporadic cases were allowed for by setting the penetrance for normal homozygotes to 0.005. For the DOMSS model these penetrance values were set to 0.76 and 0.01, respectively. Analyses for both models were carried out assuming dominant transmission with the gene frequency of the disease allele being set to 0.0085. To investigate the possibility that only a subset of pedigrees might have a susceptibility locus in the region studied, the lod2 statistic was used [Risch, 1989]. This is the log of the ratio of the likelihoods under the assumption that a proportion of families is linked at a certain recombination fraction against the likelihood under the assumption that the recombination fraction is 0.5 between disease and marker in all families. This lod2 statistic is thus similar to a lod score but includes an extra degree of freedom for α, the proportion of families which are linked. Analysis was also carried out using the MFLINK [Curtis and Sham, 1995] program which does not require specification of a particular mode of transmission. It compares the likelihoods for the observed data under the hypothesis that a locus at a particular test position influences susceptibility in a proportion of families and under the hypothesis that it has no effect. Both likelihoods are maximized independently over a wide range of transmission models and the likelihood under linkage is additionally maximised over α, the proportion of families linked. The difference between the two maximized log likelihoods provides the "model-free" lod score for the position tested. One position was tested, at recombination fraction of 0.01 with the SLC1A5 glutamate/aspartate transporter gene. MFLINK also reports the maximum lod scores obtained for any transmission model under the assumptions of homogeneity or admixture.

RESULTS

The total two-point lod scores obtained with each affection model (DOMS and DOMSS) against the SCL1A5 gene are shown in Table I. It can be seen that the results are very similar for each affection model and that under the assumption of homogeneity the total lod scores are quite negative and result in an exclusion (lod score less than -2) up to a recombination fraction of 20%. To investigate the possibility that only a subset of pedigrees might have a susceptibility locus in the region studied, the lod2 statistic was used [Risch, 1989]. The lod2 statistic did not rise above zero for any value of α or θ. The results from MFLINK are also negative with a model-free lod score of 0.00 for the DOMS and

TABLE I. Total Two-Point Lod Scores between Schizophrenia and the SLC1A5 Locus in 23 Families at Specified Recombination Fractions, Theta*

	θ						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
DOMS	−20.59	−16.35	−10.22	−6.62	−2.83	−1.02	−0.21
DOMSS	−23.49	−19.71	−12.45	−7.90	−3.32	−1.31	−0.41

* DOMS denotes the core schizophrenia model. DOMSS denotes the schizophrenia spectrum model.

DOMSS models, and an admixture lod score maximised over transmission models of only 0.00 for the DOMS model and 0.23 for the DOMSS model. These results do not support the hypothesis that the SLC1A5 allelic variants contribute to the etiology of schizophrenia.

DISCUSSION

The results obtained are valid only for the family sample used, but they provided evidence against the hypothesis that a common susceptibility locus for schizophrenia exists in the region of the gene encoding the neutral amino acid transporter; SLC1A5. It is well known that false-negative lod scores can be obtained in linkage analysis by misspecifying the mode of transmission, however, we do not consider this likely since we have also used a model-free method of analysis (MFLINK). Because of the uncertain degree to which locus heterogeneity exists in schizophrenia, we cannot rule out the possibility that SLC1A5 does exert an effect in some families and we would recommend that this locus be studied further in other families.

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